Description of *Actinomycetospora chibensis* sp. nov., *Actinomycetospora chlora* sp. nov., *Actinomycetospora cinnamomea* sp. nov., *Actinomycetospora corticicola* sp. nov., *Actinomycetospora lutea* sp. nov., *Actinomycetospora straminea* sp. nov. and *Actinomycetospora succinea* sp. nov. and emended description of the genus *Actinomycetospora*

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Eight actinomycete strains that form bud-like spore chains were isolated from various samples collected in Japan. Phylogenetically, the isolates formed a single clade with the type strain of *Actinomycetospora chiangmaiensis* according to 16S rRNA gene sequence analysis. The isolates contained meso-diaminopimelic acid, D-glutamic acid and D- and L-alanine in the cell-wall peptidoglycan, arabinose and galactose as characteristic sugars, phosphatidylcholine and phosphatidylethanolamine as diagnostic phospholipids, MK-8(H₄) as the predominant isoprenoid quinone, iso-C₁₆:₀ as the major cellular fatty acid and DNA G+C contents of 72–74 mol%. *Actinomycetospora chiangmaiensis*, the type species of the genus *Actinomycetospora*, was also found to contain MK-8(H₄) predominantly in our study, although it was earlier reported to contain MK-9(H₄) as the predominant isoprenoid quinone. On the basis of the morphological, physiological, chemotaxonomic, phylogenetic and DNA–DNA hybridization data, we concluded that the isolates can be accommodated in the genus *Actinomycetospora* with emendation of the description of the genus and are assigned to the following seven novel species: *Actinomycetospora chibensis* sp. nov. (type strain TT04-21T = NBRC 103694T = KACC 14256T), *Actinomycetospora chlora* sp. nov. (type strain TT07I-57T = NBRC 105900T = KACC 14252T), *Actinomycetospora cinnamomea* sp. nov. (type strain TT07I-57T = NBRC 105527T = KACC 14250T), *Actinomycetospora corticicola* sp. nov. (type strain 014-5T = NBRC 103689T = KACC 14253T), *Actinomycetospora lutea* sp. nov. (type strain TT00-04T = NBRC 103690T = KACC 14254T), *Actinomycetospora straminea* sp. nov. (type strain TT02-19T = NBRC 105528T = KACC 14251T) and *Actinomycetospora succinea* sp. nov. (type strain TT00-49T = NBRC 103691T = KACC 14255T).

The genus *Actinomycetospora* was proposed by Jiang *et al.* (2008) to accommodate actinomycetes that form short spore chains on the substrate mycelium and contain meso-diaminopimelic acid (*meso*-A₂pm), arabinose and galactose in the cell wall (cell-wall chemotype IV), phosphatidylcholine as a diagnostic phospholipid and MK-9(H₄) as a predominant menaquinone. At present, the only member...
of the genus is the type species Actinomycetospora chiangmaiensis, which was originally isolated from soil of a tropical rainforest in northern Thailand (Jiang et al., 2008).

During the course of an ecological study of actinomycetes in nature, eight actinomycete strains were isolated from soil and bark samples collected from subtropical islands and temperate areas of Japan (Table 1). All strains were isolated by the yeast extract-SDS method (Hayakawa & Nonomura, 1989) using humic acid-vitamin (HV) agar medium (Hayakawa & Nonomura, 1987). A. chiangmaiensis NBRC 104400T was used as a reference strain in our studies.

The 16S rRNA gene was amplified by PCR and sequenced as described previously (Tamura & Hatano, 2001). Phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). 16S rRNA gene sequences were aligned with published sequences retrieved from the DNA Data Bank of Japan (DDBJ) using CLUSTAL_X (Thompson et al., 1997), and sequences were edited manually using BioEdit version 7.0.9 (Hall, 1999). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) algorithms using the MEGA 4.1 program (Tamura et al., 2007). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replications.

16S rRNA gene sequence analyses revealed that the isolated strains formed a monophyletic cluster with A. chiangmaiensis YIM 0006T that had 100 % bootstrap support (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The binary similarity between strain 014-5T and A. chiangmaiensis YIM 0006T was 98.8 %, and similarities with other isolates ranged from 97.3 to 97.9 %. The similarity among our isolates, except strain 014-5T, ranged from 98.2 to 100 %. The 16S rRNA gene sequences of strains TT00-04T and TT01-72 were 100 % similar. All eight isolates and A. chiangmaiensis YIM 0006T showed 95.1 % similarity or less to Pseudonocardia strains.

For chemotaxonomic analyses, cells were grown in yeast extract-glucose broth (Hatano et al., 2003) on a rotary shaker at 28 °C for 5 days. Whole-cell sugar patterns, cell-wall amino acids, menaquinones, the acyl type of peptidoglycan, mycolic acids and DNA base compositions were analysed as described previously (Tamura et al., 1994). The isoprenoid quinone was determined by using liquid chromatography/mass spectrometry (LC/MS) according to Shimadzu Application Data Sheet no. 010 (Shimadzu, 2010). Cellular fatty acid methyl esters were prepared and analysed according to the protocol of the MIDI Sherlock Microbial Identification System (Sasser, 1990; MIDI, 2002). The eight isolates and A. chiangmaiensis NBRC 104400T contained MK-8(H4) as the predominant quinone. Arabinose, galactose and glucose were present as whole-cell sugars. The cell-wall peptidoglycan contained meso-A2pm, D- and L-alanine and D-glutamic acid. The major fatty acid present in these strains was iso-C16:0. The cellular fatty acid compositions of the isolates are shown in Supplementary Table S1. The phospholipids detected were phosphatidylethanolamine, phosphatidylylycerol and diphasphatidylglycerol (phospholipid type IIIs sensu Lechevalier et al., 1977). The DNA G+C content of the isolates ranged from 72.8 to 74.2 mol%.

Morphological characteristics were observed by scanning electron microscopy as described previously (Tamura et al., 1994). The isolates formed spore chains directly on the vegetative mycelium, which were observed to be bud-like, similar to those of A. chiangmaiensis. The spores were oval and rod-shaped with a smooth surface, 0.5–1.0 × 0.7–2.0 μm. Swollen spores (1.0–2.0 × 1.5–2.0 μm) were sometimes observed on the tips of the spore chains. Motile cells were not observed. Phylogenetic analysis, chemotaxonomic

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**Table 1. Actinomycete strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source, locality and year of isolation</th>
<th>16S rRNA gene accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycetospora chibensis sp. nov. TT04-21T</td>
<td>Paddy soil; Mobara, Chiba, Japan; 2004</td>
<td>AB514517</td>
</tr>
<tr>
<td>Actinomycetospora chlora sp. nov. TT071-57T</td>
<td>Paddy soil; Iriomote Island, Okinawa, Japan; 2007</td>
<td>AB514519</td>
</tr>
<tr>
<td>Actinomycetospora cinnamonoea sp. nov. IY07-53T</td>
<td>Paddy soil; Iriomote Island; 2007</td>
<td>AB514520</td>
</tr>
<tr>
<td>Actinomycetospora corticicola sp. nov. 014-5T</td>
<td>Cortex of mangrove tree (Kandelia candel); Iriomote Island; 1996</td>
<td>AB514513</td>
</tr>
<tr>
<td>Actinomycetospora lutea sp. nov. TT00-04T</td>
<td>Vegetable field soil; Amami Island, Kagoshima, Japan; 2000</td>
<td>AB514515</td>
</tr>
<tr>
<td>Actinomycetospora straminea sp. nov. IY07-55T</td>
<td>Pasture soil; Iriomote Island; 2001</td>
<td>AB514516</td>
</tr>
<tr>
<td>Actinomycetospora succinea sp. nov. TT00-49T</td>
<td>Paddy soil; Iriomote Island; 2007</td>
<td>AB514518</td>
</tr>
<tr>
<td>Actinomycetospora chiangmaiensis NBRC 104400T</td>
<td>Vegetable field soil; Amami Island; 2000</td>
<td>AB514514</td>
</tr>
<tr>
<td>TT01-72</td>
<td>Rainforest soil; Thailand; NK</td>
<td>AM398646</td>
</tr>
</tbody>
</table>

NK, Not known.
Seven novel species of the genus *Actinomycetospora*

The microplate hybridization method developed by Ezaki et al. (1988, 1989) was applied to determine DNA–DNA relatedness. The relatedness among *A. chiangmaensis* NBRC 104400T and strains 014-5T, IY07-53T, TT07I-57T, TT00-49T, TT00-04T, IY07-55T and TT04-21T ranged from 2 to 38 % (Supplementary Table S3).

The isolates displayed morphological and chemotaxonomic characteristics similar to those of *A. chiangmaensis* NBRC 104400T. In the paper that originally described the genus *Actinomycetospora*, the predominant menaquinone was reported to be MK-9(H₄) (Jiang et al., 2008). However, in our study, the predominant quinone of *A. chiangmaensis* NBRC 104400T was MK-8(H₄) rather than MK-9(H₄). The predominant quinone detected in all of our isolates was also MK-8(H₄). Therefore, it is concluded that the predominant quinone of the genus *Actinomycetospora* is MK-8(H₄). In addition, phosphatidylethanolamine as well as phosphatidylcholine was detected in all strains associated with *Actinomycetospora*.

DNA–DNA relatedness between strains TT00-04T and TT01-72 was 63 % (reciprocal value 83 %). Strains TT00-04T and TT01-72 were therefore identified as members of the same species. The chemotaxonomic characteristics as well as 16S rRNA gene sequence analyses show unambiguously that these isolates are affiliated with the genus *Actinomycetospora*. Phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences indicate clearly that these isolates represent seven novel species belonging to the genus *Actinomycetospora* (Table 2). The chemotaxonomic analyses show unambiguously that these isolates are affiliated with the genus *Actinomycetospora*. Phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences indicate clearly that these isolates represent seven novel species belonging to the genus *Actinomycetospora*.

**Table 2.** Differential characteristics of the eight novel isolates and *A. chiangmaensis* NBRC 104400T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pyrrolidonyl arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Esterase (C-4)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Valine aminopeptidase</td>
<td>W</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>W</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythitol</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>V</td>
<td>−</td>
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<td>Trehalose</td>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Turanose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>l-Arabinol</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>V</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Growth pH</td>
<td>6–7</td>
<td>5–8</td>
<td>5–8</td>
<td>5–9</td>
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<td>5–8</td>
<td>6–8</td>
<td>5–9</td>
<td>ND</td>
</tr>
<tr>
<td>Tolerance of NaCl (% w/v)</td>
<td>0–1</td>
<td>0–1</td>
<td>0–7</td>
<td>0–7</td>
<td>0–1</td>
<td>0–1</td>
<td>0–5</td>
<td>0–7</td>
<td>ND</td>
</tr>
</tbody>
</table>

The isolates displayed morphological and chemotaxonomic characteristics similar to those of *A. chiangmaensis* NBRC 104400T. In the paper that originally described the genus *Actinomycetospora*, the predominant menaquinone was reported to be MK-9(H₄) (Jiang et al., 2008). However, in our study, the predominant quinone of *A. chiangmaensis* NBRC 104400T was MK-8(H₄) rather than MK-9(H₄). The predominant quinone detected in all of our isolates was also MK-8(H₄). Therefore, it is concluded that the predominant quinone of the genus *Actinomycetospora* is MK-8(H₄). In addition, phosphatidylethanolamine as well as phosphatidylcholine was detected in all strains associated with *Actinomycetospora*.

DNA–DNA relatedness between strains TT00-04T and TT01-72 was 63 % (reciprocal value 83 %). Strains TT00-04T and TT01-72 were therefore identified as members of the same species. The chemotaxonomic characteristics as well as 16S rRNA gene sequence analyses show unambiguously that these isolates are affiliated with the genus *Actinomycetospora*. Phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences indicate clearly that these isolates represent seven novel species belonging to the genus *Actinomycetospora* (Table 2). The chemotaxonomic analyses show unambiguously that these isolates are affiliated with the genus *Actinomycetospora*. Phenotypic differences, the results of the DNA–DNA pairing studies and differences in 16S rRNA gene sequences indicate clearly that these isolates represent seven novel species belonging to the genus *Actinomycetospora*.

**Table 2.** Differential characteristics of the eight novel isolates and *A. chiangmaensis* NBRC 104400T

| Strains: 1, *A. chibensis* sp. nov. TT04-21T; 2, *A. chlora* sp. nov. TT07I-57T; 3, *A. cinnamomea* sp. nov. IY07-53T; 4, *A. corticicola* sp. nov. 014-5T; 5, *A. lutea* sp. nov. TT00-04T; 6, *A. succinea* sp. nov. TT01-72; 7, *A. straminea* sp. nov. IY07-55T; 8, *A. succinea* sp. nov. TT00-49T; 9, *A. chiangmaensis* NBRC 104400T. +, Positive; −, negative; w, weakly positive; V, variable; ND, not determined. Data were obtained in this study. |
sp. nov. (type strain TT00-04T; reference strain TT01-72), Actinomycetospora straminea sp. nov. (type strain IY07-55T) and Actinomycetospora succinea sp. nov. (type strain TT00-49T).

**Emended description of the genus Actinomycetospora Jiang et al. 2008**

The description of the genus is as given by Jiang et al. (2008), with the following modifications. The predominant menaquinone is MK-8(H4). Phosphatidylcholine and phosphatidylethanolamine are the diagnostic phospholipids.

**Description of Actinomycetospora chibensis sp. nov.**

*Actinomycetospora chibensis* (chi.ben’sis. N.L. fem. adj. chibensis of or belonging to Chiba, a Japanese prefecture, from where the type strain originated).

The surface of colonies is powdery. The colour of the substrate mycelium is vivid to light yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 15–28 °C, pH 6.0–7.0 and NaCl concentrations up to 1 %. D-Arabinitol, aesculin ferric citrate, glycerol, 2-ketogluconate, 5-ketogluconate, D-lyxose, D-mannitol, L-rhamnose, D-ribose, D-sorbitol, starch and turanose are utilized as sole carbon sources. Positive for nitrate reduction, gelatin hydrolysis, alkaline phoshatase, esterase (C-8), esterase lipase (C-8), α-glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for aesculin hydrolysis, urea hydrolysis and nitrate reduction. The major cellular fatty acids are iso-C\textsubscript{16:0} and C\textsubscript{17:1} cis-9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT07I-57\textsuperscript{T} (=NCPC 305900\textsuperscript{T} =KACC 14252\textsuperscript{T}), which was isolated from a paddy soil on Iriomote Island, Okinawa, Japan.

**Description of Actinomycetospora cinnamomea sp. nov.**

*Actinomycetospora cinnamomea* (cin.na.mo’e.a. L. n. cinnamomum cinnamon; L. fem. suff. -a suffix used with various meanings; N.L. fem. adj. cinnamomea cinnamon-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is orange. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 15–37 °C, pH 5.0–8.0 and NaCl concentrations up to 7 %. D-Arabinitol, D-fructose, glycerol, D-mannose, D-mannitol and D-sorbitol are utilized as sole carbon sources. Positive for gelatin hydrolysis, acid phosphatase, alkaline phosphatase, esterase (C-4), esterase lipase (C-8), α-glucosidase, leucine aminopeptidase and pyrazinamidase, but negative for aesculin hydrolysis and urea hydrolysis. The major cellular fatty acids are iso-C\textsubscript{16:0} and C\textsubscript{17:1} cis-9. The DNA G+C content of the type strain is 74 mol%.

The type strain is IY07-53\textsuperscript{T} (=NBRC 105527\textsuperscript{T} =KACC 14250\textsuperscript{T}), which was isolated from paddy soil on Iriomote Island, Okinawa, Japan.

**Description of Actinomycetospora corticicola sp. nov.**

*Actinomycetospora corticicola* [cor.ti.ci’co.la. L. n. cortex -icis the bark; L. suff. -cola (from L. n. incola) inhabitant, dweller; N.L. n. corticicola inhabitant of bark].

The surface of the colonies is powdery. The colour of the substrate mycelium is orange–yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 10–37 °C, pH 5.0–9.0 and NaCl concentrations up to 7 %. D-Arabinitol, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, melezitose, D-sorbitol, sucrose, trehalose, turanose and xylitol are utilized as sole carbon sources. Positive for aesculin hydrolysis, gelatin hydrolysis, urea hydrolysis, acid phosphatase, alkaline phosphatase, esterase lipase (C-8), α-glucosidase, leucine aminopeptidase, pyrazinamidase, pyrrolidonyl arylamidase and valine aminopeptidase, but negative for nitrate reduction. The major cellular fatty acids are iso-C\textsubscript{16:0} and 10-methyl C\textsubscript{16:0}. The DNA G+C content of the type strain is 74 mol%.

The type strain is 014-5\textsuperscript{T} (=NBRC 103694\textsuperscript{T} =KACC 14256\textsuperscript{T}), which was isolated from a paddy field soil in Mobara, Chiba, Japan.
Description of Actinomycetospora lutea sp. nov.

Actinomycetospora lutea (lu’t’e.a. L. fem. adj. lutea yellow-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is vivid to light yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 20–37 °C and pH 5.0–8.0. D-Arabitol, D-fructose, D-glucose, D-mannitol, D-sorbitol and turanose are utilized as sole carbon sources. Positive for gelatin hydrolysis, acid phosphatase, esterase (C-4), esterase lipase (C-8), α-glucosidase, leucine aminopeptidase, phosphohydrolase and pyrazinamidase, but negative for aesculin hydrolysis, nitrate reduction and urea hydrolysis. The major cellular fatty acids are iso-C_{16:0}, C_{17:1} ω9, C_{15:0} and C_{16:1} ω9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT00-49^T (=NBRC 103691^T =KACC 14255^T), which was isolated from vegetable field soil on Amami Island, Kagoshima, Japan. Strain TT01-72 (=NBRC 103692), from a similar source, is a second strain of the species.

Description of Actinomycetospora straminea sp. nov.

Actinomycetospora straminea (stra.mi’ne.a. L. fem. adj. straminea made of straw, intended to mean straw-coloured).

The surface of the colonies is powdery. The colour of the substrate mycelium is pale greenish yellow to yellow. Spore chains are white and the spore surface is smooth. The substrate mycelium is long, well-developed and branched. Grows at 20–37 °C and pH 5.0–8.0. D-Adonitol, D-arabitol, erythritol, D-fructose, D-galactose, D-glucose, glycerol, maltose, D-mannitol, D-mannose, D-sorbitol, sucrose, trehalose and xylitol are utilized as sole carbon sources. Positive for gelatin hydrolysis, urea hydrolysis, esterase lipase (C-8), α-glucosidase, leucine aminopeptidase, pyrazinamidase and valine aminopeptidase, but negative for aesculin hydrolysis and nitrate reduction. The major cellular fatty acids are iso-C_{16:0} and C_{17:1} ω9. The DNA G+C content of the type strain is 73 mol%.

The type strain is TT00-49^T (=NBRC 103691^T =KACC 14255^T), which was isolated from vegetable field soil on Amami Island, Kagoshima, Japan.

Acknowledgements

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